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Procyanidolic oligomers enhance photodegradation of chlorothalonil in water *via* reductive dechlorination

Pei Lv[a], Jun Zhang[a], Taozhong Shi[a], Leilei Dai[a], Xiangqiong Li[a], Xiangwei Wu[a], Xuede Li[a], Jun Tang[a], Yi Wang[a], Qing X. Li[a,b], Rimao Hua[a,*]

[a] Key Laboratory of Agri-Food Safety of Anhui Province, School of Resource & Environment, Anhui Agricultural University, Hefei, Anhui 230036, China
[b] Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, 1955 East-West Road, Honolulu, HI 96822, United States

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ABSTRACT

Chlorothalonil is an important broad-spectrum fungicide with an annual application rate of above ten thousands of tons of its active ingredient on agricultural crops world-wide. The effect of procyanidolic oligomers on photo degradation of chlorothalonil was investigated under sunlight and artificial lights. Procyanidolic oligomers enhanced photodegradation of chlorothalonil in paddy, reservoir, pond and distilled waters for 1.8, 4.6, 2.7 and 22.8 fold, respectively, relative to the procyanidolic oligomers free control. The mechanism was evidenced as a radical reduction reaction by electron paramagnetic resonance spectroscopy. Upon exposure to high-pressure mercury light, chlorothalonil produced 2,4,5-trichloro-1,3-dicyanobenzene, 2,5-dichloro-1,3-dicyanobenzene and 5-chloro-1,3-dicyanobenzene that were isolated, identified and characterized. Chlorothalonil underwent primarily step-wide photo reductive dechlorination in the presence of procyanidolic oligomers, which avoided the production of the highly toxic 4-hydroxy chlorothalonil. Procyanidolic oligomers possessed strong reductive property to photo reductive dechlorination. The results contributed to understanding of chlorothalonil phototransformation and high potential of using natural product procyanidolic oligomers as an additive to minimize aquatic toxicity and pollution of chlorothalonil.

1. Introduction

Chlorothalonil (2,4,5,6-tetrachloro-1,3-dicyanobenzene, CTL) is a broad-spectrum, nonsystemic fungicide commonly applied on a large number of agricultural crops, especially vegetables and fruits. The annual application rate of its active ingredient was approximately ten thousands of tons on agricultural crops world-wide. Owing to its wide use and persistence in the environment, CTL is commonly detected in vegetables and fruits [1,2], soil and surface water [3,4], ground water and greenhouse air [5,6]. In addition, CTL has been used as an alternative to tributyltin in antifouling paints to prevent the growth of fouling organisms [7]. Since CTL and its metabolites are highly toxic to fish, aquatic invertebrates [8] and amphibians [9], as well as it could cause serious marine pollution impacting a number of marine species other than the fouling organisms [10], CTL pollution is of concern.

The main metabolites of CTL in aquatic environments include 4-hydroxy chlorothalonil (4-OH-CTL) [11] and other degradation products, which are derived from dechlorination [12,13] and oxidation/hydration [14,15]. To remediate CTL toxicity to aquatic species, many chemical treatments [15–20] were investigated to accelerate the degradation of CTL utilizing oxidation process. The feature of such treatments was generation of hydroxyl radicals giving rise to induce photodegradation which leads to the production of 4-OH-CTL. Unfortunately, 4-OH-CTL is approximately 30 times more toxic than its parent compound CTL. The acute oral LD_{50} values of 4-OH-CTL and CTL on rat are 332 and 10,000 mg/kg, respectively [21]. In addition, 4-OH-CTL is more persistent than CTL in the environment [20]. Therefore, methods to enhance CTL degradation and generate less toxic products in the environment are needed to manage CTL aquatic pollution. In our previous study, the natural product epigallocatechin gallate (EGCG) was used to promote the degradation of CTL [22], but the mechanism and the pathway of photodegradation were not studied.

In our continuous efforts to search for a natural product possessing stronger reducing power in comparison to EGCG, procyanidolic oligomers (OPC) were investigated for roles on pho-

* Corresponding author.
E-mail addresses: rimaohua@ahau.edu.cn, rimaohua@aliyun.com (R. Hua).

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tolysis efficiency, photodegradation pathways and mechanisms of chlorothalonil in aqueous solutions. OPC are the second most abundant plant polyphenolic compounds after lignin which could be found in most plants, especially epidermis, seeds, and seed coats of purple or red pigmented plants [23]. Additionally, OPC can be produced *via in vitro* cell culture of *Vaccinium pahalae* [24]. OPC have attracted increasing attention due to their potential health benefits such as antioxidant activity and the ability to scavenge reactive oxygen and nitrogen species. It has been hypothesized that the free radical scavenging properties of OPC may reduce the risk of cardiovascular diseases and cancer [25,26]. As OPC is the economical source and nontoxicity to human, it is significant to study OPC's reduction potential to photodegrade chlorothalonil as it represents its general value of such enhanced degradation in the environment.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Chem Service and used as received. Chlorothalonil (99.1%), 4-OH-CTL (99%), OPC (95%), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO, 97%) and *N*,*N*-dimethyl-*p*-nitrosoaniline (PNDA, 98%) were purchased from J&K Chemical Ltd. (Shanghai, China). Acetonitrile (Assay ≥99.9%, HPLC/Spectro) and methanol (Assay ≥99.9%, HPLC/Spectro) were purchased from Tedia Co. (Fairfield, USA).

2.2. Photodegradation experiments

The photocatalytic degradation experiments were conducted under sunlight and a rotary photochemical reactor equipped with a 150 W high-pressure mercury lamp (HPML, emission line spectrum at 365 nm). An electric fan and cycled condensate water were used to control the temperature and to prevent the thermal catalytic effects. The speed of quartz tube surrounding light source in the photochemical reactor was 4 r/min. The distance between the quartz tube and the light source was 15 cm. The light intensity was 10,000–11,000 Lx for a high-pressure mercury lamp, and 84,000–93,000 Lx for direct sunlight (N31°52′, E117°17′). The temperature of the reaction system was maintained at 25 ± 1 °C except for the sunlight treatment which temperatures varied between 25 °C and 30 °C. A rotary water bath was used to maintain the temperature and the quartz tube was tilted at a 30° angle with the ground to face the sun directly. CTL standard stock solution was 1.0 g/L (3.76 mmol/L) in acetonitrile. The concentration of OPC was prepared at 5.0 g/L. To determine the effect of OPC on photodegradation of CTL in aqueous solution, CTL standard stock solution and OPC solution were transferred into 100 mL flasks at a mole to mole ratio (CTL:OPC) of 1:0, 1:1.8, 1:4.5, 1:9.0, 1:22.5, and 1:45 (CTL was 1.88 μmol/L). To the flasks, double-distilled water was added and subjected to brief ultrasonication. An aliquot of 10 mL reaction solution of CTL and OPC was transferred to a quartz cuvette that was covered with a stopper and placed under different light sources.

Paddy, reservoir and pond waters (Table 1) were used to determine the effect of OPC on photodegradation of CTL in natural water. To a quartz cuvette, an aliquot of 10 mL reaction solution of CTL and OPC (CTL:OPC of 1:9.0, CTL is 1.88 μmol/L) was transferred, then covered with a stopper and placed under sunlight. The quartz tube was tilted at a 30° angle with the ground to face the sun directly. The light intensity was 84,000–93,000 Lx for direct sunlight (N31°52′, E117°17′), and the temperature of the water solution varied between 25 °C and 30 °C.

2.3. Photodegradation of CTL and analysis of 4-OH-CTL in distilled water

An appropriate amount of CTL standard stock solution was transferred to a 100 mL volumetric flask and OPC was added at a mole to mole ratio of 1:9 (CTL is 1.88 μmol/L). The mixture solution was diluted with double-distilled water and subjected to brief ultrasonication. Controls contained no OPC. An aliquot of 20 mL of the mixture was transferred to a quartz tube with a stopper and exposed to HPML. Afterwards, samples were taken at different times to measure the concentrations of CTL and chloride ion. The flasks completely covered with aluminum foil were used as a dark control, while the other conditions remained the same as the treatments. The samples were taken at different time intervals and each treatment was repeated twice. After incubation, the photoreaction mixtures were filtered through a 0.45 μm membrane filter. The concentrations of CTL and 4-OH-CTL in the filtrate were determined and analyzed immediately by HPLC or stored at 4 °C.

Quantitative analysis of CTL and 4-OH-CTLwere performed on an Agilent 1200 HPLC system equipped with an Agilent HC-C18 column (4.6 mm × 250 mm, 5 μm) at a detection wave length of 236 nm and 248 nm, respectively (Agilent Technologies, USA). CTL and 4-OH-CTL were respectively eluted with 80% aqueous acetonitrile and a mixture of acetonitrile/0.5% phosphoric acid solution (60:40 v/v) at a flow rate of 1.0 mL/min. The column temperature was 30 °C. The injection volume was 10 μL or 20 μL. The limit of detection for CTL and 4-OH-CTL was 0.01 mg/L.

2.4. Detection of hydroxyl free radicals (•OH) and chloride ion

Hydroxyl free radicals (•OH) and chloride ion were determined by UV spectro photometer and ion chromatograph according to previously published methodologies [22].

2.5. Analysis of the other photolytic products of CTL

The mixture of photolytic products of CTL was partitioned with 20 mL of methylene chloride using a separatory funnel three times. After organic solvent extracts were combined, methylene chloride was evaporated with a rotary evaporator at 40 °C in a water bath. The residues were dissolved in 10 mL of acetone. The samples were then analyzed on an Agilent GC–MS (7890A-5975) system equipped with a HP-5 (30m × 0.25mm × 0.25 μm) column. The oven temperature was started at 55 °C, ramped at 5 °C/min to 200 °C followed by another ramp of 1 °C/min to 210 °C, held for 2 min and finally to 270 °C at 20 °C/min (held for 3 min). Helium was used as carrier gas at a constant flow of 1 mL/min. The temperatures of the injector, ion source and the interface were set at 240, 240 and 290 °C, respectively. The MS was operated in electron impact mode with an ionization potential of 70 eV and the spectra were obtained at a scan range from *m*/*z* 50 to 450 (selective scan mode). The scan time was 50 min. The injection volume was 3.0 μL.

Table 1
The property of paddy, reservoir, and pond waters.

Natural Water	Turbidity FTU	Conductivity ms/cm	pH	Hardness mg/L	DO mg/L	COD mg/L	BOD_5 mg/L	TOC mg/L	Cl^- mg/L	SO_4^{2-} mg/L	NO_2^- mg/L
Paddy	1.8	0.29	7.42	264	4	342	2.4	15.08	46.4	75.9	0.59
Reservoir	1.4	0.15	7.09	124	15.2	8	6.4	9.62	14.6	27.9	0.53
Pond	2.3	0.32	7.38	140	4.8	72	2.4	27.82	52.1	72.5	0.57

Table 2
Effects of OPC on photodegradation of chlorothalonil (1.88 μM) in water[a] under sunlight and HPML irradiation.[b]

Light source[c]	Mole ratio (CTL/OPC)	Kinetic equations		$T_{1/2}$ (min)
		K/min^{-1}	R^2	
Sunlight	1: 0	0.0038	0.9939	184.6 (±4.61)
	1:1.8	0.042	0.9905	16.2 (±0.52)
	1:4.5	0.077	0.9888	10.2 (±0.31)
	1:9.0	0.1146	0.9749	8.1 (±0.21)
	1: 22.5	0.1423	0.9952	5.3 (±0.16)
	1:45	0.2043	0.9982	3.1 (±0.1.4)
HPML	1: 0	0.0044	0.9921	161.2 (±3.7)
	1:1.8	0.0234	0.9957	31.4 (±1.9)
	1:4.5	0.0411	0.9884	16.9 (±0.77)
	1:9.0	0.0545	0.9918	13.6 (±0.38)
	1: 22.5	0.0622	0.9976	9.9 (±0.25)
	1:45	0.0857	0.9883	8.5 (±0.33)

[a] Distilled water.
[b] Number of each degradation experiment, n = 3.
[c] No degradation of CTL with OPC under dark condition in water.

Table 3
Effects of OPC on generation of Cl^- from CTL (1.88 μM) in water under HPML irradiation.[a]

Experimental group	Molar ratio (μM)	Concentrations of Cl^- (μM) at different irradiation times (min)		Percent CTL photodegraded
		0	240	
Double-distilled water		ND[b]	ND	
OPC/CTL	0:1.88	ND	2.71 ± 0.07	72 ± 2.1
OPC/CTL	16.92:1.88	ND	5.43 ± 0.09	100 ± 0.5

[a] Number of each degradation experiment, n = 3.
[b] Not detected.

The photodegradation mixture of CTL in a quartz tube with 20 mL of double-distilled water in the presence of OPC at a mole to mole ratio of 1:9.0 (CTL is 1.88 μmol/L) was extracted with petroleum ether/ethyl acetate (volume: 1:1) and concentrated. The photodegradation products were isolated with a Waters 2695/2998 series instrument equipped with a Prontosil-120-3-C18-ace-EPS (3 μm), 150 × 4.6 mm column and were detected at 254 nm or 230 nm or 210 nm absorption. NMR spectra were obtained on an Agilent 600 M DD2 spectrometer using DMSO-d_6 as solvent. The chemical shifts were expressed in ppm with DMSO-d_6 as the internal standard. Electron paramagnetic resonance (EPR) analysis data were obtained on an electron spin (paramagnetic) resonance spectrometer JES-FA200 (JEOL Ltd., Tokyo, Japan). The operating conditions were as follows: magnetic field 336.5 ± 6.0 mT width, power attenuation 10 dB, field modulation 0.100 mT, sweep time 100 s, microwave frequency 9450 MHz.

2.6. Calculation methods

Photolysis rate was calculated with Eq. (1):

$$\text{photolysisrate}(\%) = [(a-b)/a] \times 100 \quad (1)$$

where a was the residual concentration of CTL in the dark control and b was the residual concentration of CTL in the light treatment.

Photodegradation half-life ($T_{1/2}$) was defined as the time required for the residue concentration to fall to half of the initial level and calculated from the k value for each experiment calculated with Eq. (2):

$$T_{1/2} = \ln 2/k \quad (2)$$

where k was the photolysis rate constant and was calculated with Eq. (3):

$$C_t = C_0 \cdot e^{-kt} \quad (3)$$

where C_0 and C_t are residual concentrations at time zero and time t after the light treatment, respectively.

Photolysis efficiency was calculated with an Eq. (4):

$$\text{photolysisefficiency}(\%) = [(k_1 - k_0)/k_0] \times 100 \quad (4)$$

where k_1 and k_0 are the reaction rate constant of CTL with OPC and that of CTL without OPC, respectively.

2.7. Toxicological experiments

The acute toxicity of zebrafish was conducted under the strict control of the Guiding Principles outlined in the Use of Animals in Toxicology which were adopted by the Society of Toxicology. Briefly, each tank contained 10 fish of a mixture of male and female adult zebrafish (Danio rerio) at a ratio of 1:1. There were 3 replicate tanks per concentration of CTL (0, 20, 30, 40, 50, 60, and 70 μg/L), 5-chloro-1,3-dicyanobenzene (0, 3, 6, 9, 12, 15 and 18 mg/L), and 4-OH-CTL (0, 1.2, 1.6, 2.0, 2.4, 2.8, and 3.0 mg/L), based on preliminary studies. The exposure media was prepared with dechlorinated carbon-filtered water and replaced with freshly prepared media daily. Control and experimental treatment groups received 0.01% (v/v) DMSO. All the test groups were placed at 26 ± 1 °C with a ratio of 14:10-h light: dark photoperiod and the fish were not fed during the experimental periods. The numbers of dead fish were recorded every twenty-four hours (24 h) for the duration of the entire experimental period, and the dead fish were promptly removed. The lethal median concentration (LC_{50}) value was obtained through probit analysis and the upper and lower confidence intervals were calculated using SPSS for Windows (Version 22.0; Chicago, IL, USA).

3. Results and discussion

3.1. Effects of OPC on chlorothalonil photodegradation in water

Photodegradation of chlorothalonil in distilled water was significantly enhanced in the presence of OPC in both natural sunlight and HPML irradiation (Table 2). With the concentrations of OPC increased, efficiency also increased. The enhanced photodegrada-

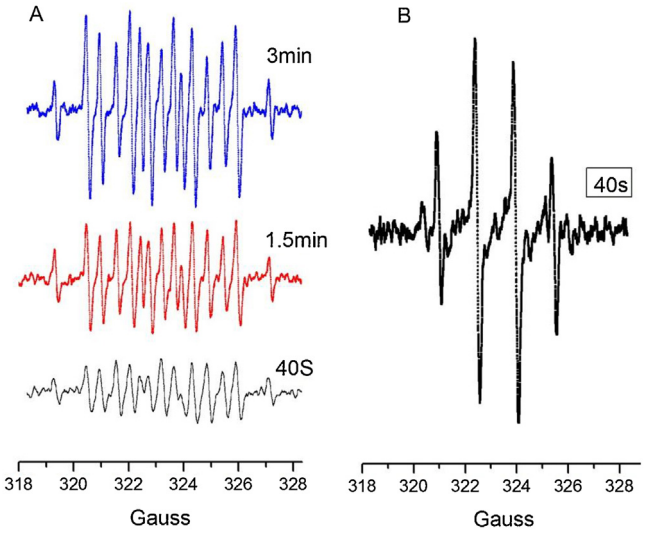

Fig. 1. A: Electron paramagnetic resonance (EPR) spectra of DMPO-phenoxyl radical spin adduct of OPC. B: EPR spectra of DMPO-OH radical spin adduct without OPC in water under HPML irradiation. Procedure: An aliquot of 10 mL reaction solution of 1.88 μmol/L CTL and 16.9 μmol/L OPC in a quartz cuvette was covered with a stopper and placed under HPML irradiation for 15 min. Then, 30 μL of the solution was transferred into a glass capillary tube by the use of a microsyringe and the EPR spectrum was recorded on a JES-FA200 spectrometer. Instrument conditions were: microwave frequency 9.43 GHz, microwavepower 2 mW, modulation frequency 100 kHz, modulation amplitude 3 G, sweep rate 2 G/s; time constant 10.24 ms, average of five sweeps for each spectrum, temperature 292 K.

tion was attributed to the presence of naturally antioxidant activity and as an H-donor of OPC in water.

The rate constant was increased 23 fold and 12 fold in both natural sunlight and HPML irradiation, respectively, at a molar ratio of CTL to OPC of 1:9.0. The photolysis enhancement by OPC was much greater than EGCG [22]. As the molar ratio of CTL:OPC increased to 1:45, the rate constant was increased 60 fold and 19 fold under sunlight and HPML irradiation, respectively, in comparison with that without OPC (Table 2).

3.2. Mechanism of enhanced effects of OPC on chlorothalonil photodegradation

To investigate the mechanism of OPC on CTL photodegradation, the concentrations of both CTL and chloride ion in the reaction mixture under HPML were determined. After irradiation for 240 min under HPML, 72% of CTL (1.36 μmol/L) was degraded (Table 3), while 2.71 μmol/L of Cl^- was detected in this solution, the initial concentration of CTL was 1.88 μmol/L. When OPC was added at a molar ratio of 9:1 OPC:CTL, CTL was completely degraded after 240 min irradiation under HPML, while 5.43 μmol/L of Cl^- was detected, suggesting an enhancement of dechlorination of CTL upon addition of OPC. The apparent increase in Cl^- production during CTL photodegradation in the presence of OPC indicated that the photocatalytic effect of OPC on CTL degradation was performed photo reductive dechlorination. The generation of phenoxy radicals from OPC was detected (Fig. 1), which demonstrated that OPC served as an H-donor. The mechanism of the reaction may be initiated by illumination, which CTL was excited to the triplet state to form the radical by a proton (OPC served as H-donor), and then dechlorination to give trichloro-1,3-dicyanobenzene. Further reductive dechlorination of trichloro-1,3-dicyanobenzene may occur to produce dichloro-1,3-dicyanobenzene and then chloro-1,3-dicyanobenzene. Other fates of the triplet state included oxidation by oxygen or reaction with hydroxyl radicals to generate the radicals which give 4-OH-CTL.

As hydroxyl free radicals (•OH) could degrade organics *via* free radical reactions, PNDA was used as a free radical trapping agent to detect •OH in order to further confirm the reduction ability of OPC. The residual concentrations of PNDA decreased apparently when H_2O_2 was added, indicating the generation of •OH from H_2O_2 (Table 4). However, when OPC were added into the mixture of PNDA and H_2O_2, the residual concentrations of PNDA did not change, suggesting that OPC can quench •OH. The results indicate OPC enhanced reductive photolysis of CTL rather than photo oxidation by •OH free radicals.

Photodegradation of chlorothalonil in the presence of OPC was also performed at the oxygen free condition and aerobic condition to further confirm the effect of OPC on CTL photodegradation *via* reductive dechlorination. After irradiation for 20 min and 40 min under HPML in the presence of OPC (OPC:CTL, molar ratio at 9:1), 95% and 100% of CTL were degraded (Table 5) at the oxygen free condition, while only 71% and 82% of CTL was photodegraded in

Table 4
Effects of hydroxyl free radical by OPC as determined with PNDA in water[a] under HPML irradiation.

Experimental group	Concentration ratio (mg/L)	Concentrations of PNDA (mg/L) at different irradiation times			
		0 min	10 min	20 min	40 min
PNDA		1.04	1.04	1.03	1.02
PNDA/H_2O_2	1:300	1.02	0.94	0.82	0.64
PNDA/CTL	1:0.5	1.03	1.03	1.02	1.01
PNDA/OPC	1:10	1.03	1.03	1.02	1.02
PNDA/OPC/H_2O_2	1:10:300	1.03	0.92	0.77	0.63
PNDA/OPC/CTL	1:10:0.5	1.05	1.04	1.03	1.03

[a] Distilled water.

Table 5
Effects of oxygen on photodegradation of chlorothalonil (1.88 μM) with OPC[a] in water[b] under HPML irradiation for varying periods of time.[c]

Experimental group	Photolysis rate of CTL (%)		Experimental group	Photolysis rate of CTL (%)
	20 min	40 min		90 min
N_2/OPC + CTL	94.7 (±0.8)	100.0 (±0.4)	N_2/CTL	74.8 (±0.3)
OPC + CTL	70.8 (±1.3)	82.2 (±1.7)	CTL	31.0 (±2.7)

[a] The mole ratio of OPC to CTL was 9:1.
[b] Distilled water.
[c] Number of each degradation experiment, n = 3.

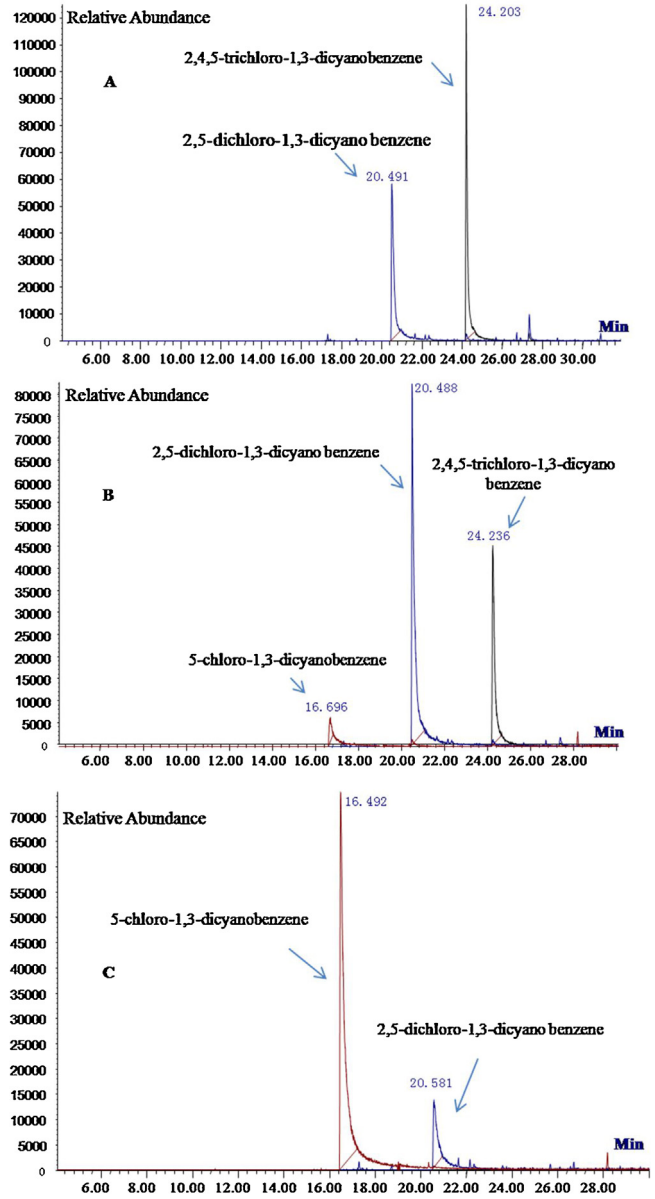

Fig. 2. Extracted ion chromatograms of photodegradation products of CTL after irradiation in the presence of OPC under HPML for 20, 40, and 240 min.

the air condition, respectively. In absence of OPC, 75% and 31% of CTL was photodegraded in the oxygen free condition and in the aerated condition after irradiation for 90 min under HPML. The results showed that oxygen prevented photodegradation of CTL. Since oxygen is a triplet quencher [12] and also could prevent the formation of hydrogen radical of OPC, photodegradation reactions of CTL by OPC are likely unfavorable in well-aerated water bodies.

3.3. Pathway of CTL photodegradation in the presence of OPC and minimizing formation of toxic metabolite

In order to identify the structure of degradation products, the three major degradation products were isolated by preparative-HPLC. Two pure products were obtained and identified as 2,5-dichloro-1,3-dicyanobenzene and 5-chloro-1,3-dicyanobenzene by ^{1}H NMR and GC–MS (Figs. S7–S10, Supplemental data). CTL possessed dicyano substituent at 1,3-position of benzene ring, which a strong electro-withdrawing effect, cleavage of the C—Cl bond at 4,6-position may favor to occur. The other compound was not stable enough during lyophilization, but it could be tentatively identified as 2,4,5-trichloro-1,3-dicyanobenzene according to its mass spectrum and further degradation product. Fig. 2 shows the GC–MS chromatograms of CTL in the presence of OPC at the different illumination times. Under HPML for 20 min, the reductive photodegradation products were 2,4,5-trichloro-1,3-dicyanobenzene as the main product, and 2,5-dichloro-1,3-dicyanobenzene, 5-chloro-1,3-dicyanobenzene was not detected. When illumination time was 40 min, 2,4,5-trichloro-1,3-dicyanobenzene, 2,5-dichloro-1,3-dicyanobenzene and 5-chloro-1,3-dicyanobenzene were detected, while 2,5-dichloro-1,3-dicyanobenzene was the main product. After illumination for 240 min, only 5-trichloro-1,3-dicyanobenzene was detected. It is noteworthy that 5-chloro-1,3-dicyanobenzene could not be further degraded under the experimental condition. According to the photoproducts identified, the proposed photodegradation pathway of CTL in the presence of OPC was shown in Fig. 3.

The effect of OPC on the production of 4-CTL-OH was studied, as 4-CTL-OH was a main photodegradation product of CTL [20]. The results showed that no 4-OH-CTL was detected in the reaction solution by comparison of their retention times and mass spectra with those of the corresponding standards when OPC was added at a level of 5 mg/L. The concentrations of 4-OH-CTL increased as the illumination time increased to 60 min without OPC. This might be explained by the strong antioxidant capacity of OPC as a polyphenol which could quench free radical reactions in the process of OPC-catalyzed CTL photodegradation.

In order to compare the toxicity of photodegradation products, laboratory bioassay was undertaken to determine the toxic effects of CTL, 4-OH-CTL, and 5-dichloro-1,3-dicyanobenzene on zebrafish. The 96-h LC_{50} values of CTL, 4-OH-CTL, and 5-dichloro-1,3-dicyanobenzene for zebrafish were 0.052 mg/L, 2.39 mg/L, and 13.1 mg/L, respectively (Table S6, Supplemental data). The results showed that 96-h LC_{50} value of photodegrdation product was 252 and 5.5 fold more than CTL and 4-OH-CTL on zebrafish, that was it was less toxic than CTL and 4-OH-CTL.

Table 6
Effects of OPC on photodegradation of chlorothalonil in natural water under sunlight irradiation.[a]

Natural water	Molar ratio[b] (CTL/OPC)	Kinetic equations		$T_{1/2}$ (min)	Photolysis efficiency of OPC (%)
		k (min^{-1})	R^2		
Reservoir water	1:0	0.0057	0.987	98	130 ± 3.4
	1:9	0.0131	0.998	21	
Pond water	1:0	0.0073	0.988	89	134 ± 5.1
	1:9	0.0171	0.990	33	
Paddy water	1:0	0.0102	0.992	57	34.3 ± 1.3
	1:9	0.0137	0.974	32	

[a] Number of each degradation experiment, n = 3.
[b] Photolysis container is glass beaker.

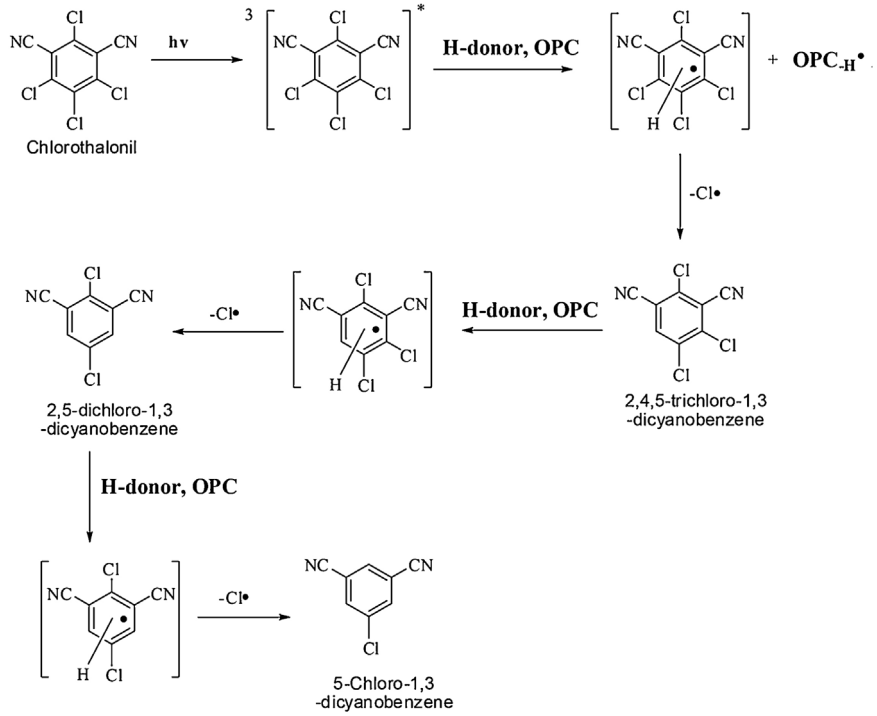

Fig. 3. Proposed mechanism of enhanced effects of OPC on chlorothalonil photodegradation.

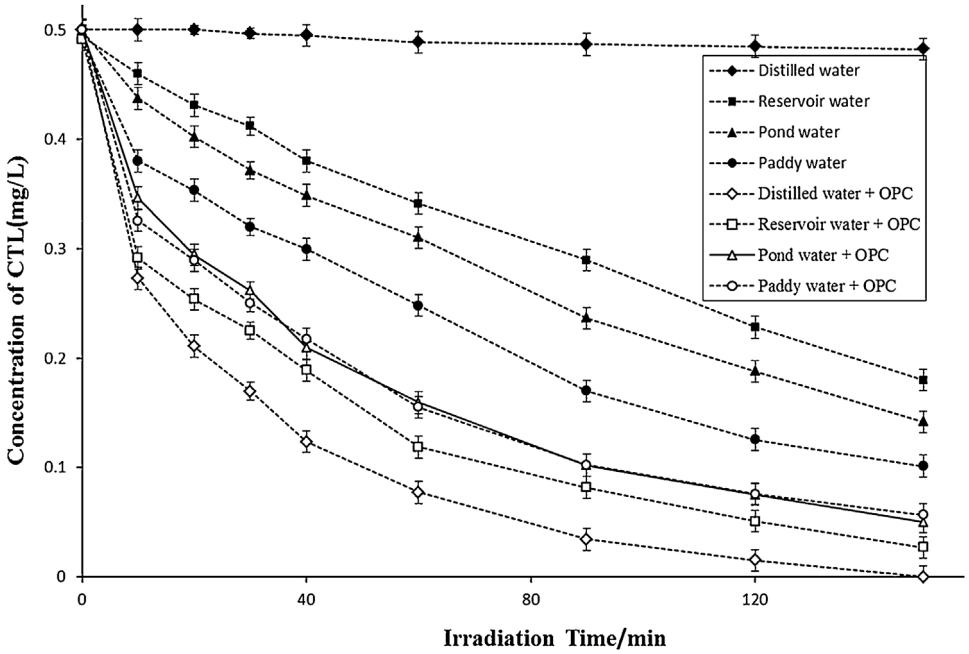

Fig. 4. Effects of OPC on photodegradation of chlorothalonil in natural water under sunlight irradiation.

3.4. *Effects of OPC on CTL photodegradation in nature water*

Under the sunlight irradiation, OPC significantly enhanced photolysis of CTL in three different types of natural waters (Fig. 4). The photodegradation half-lives of CTL in the reservoir, pond and paddy waters were 97, 89 and 57 min, respectively, while those were 21, 33 and 33 min when OPC was added (Table 6). The photodegradation half-lives of CTL were shortened 4.7, 2.7, and 1.8 fold in the reservoir, pond and paddy waters, respectively. The photolysis rate in paddy water was faster than those in reservoir and pond waters without OPC, which might be attributed to the constituents in the paddy water on triplet-induced transformation of CTL. The chemical oxygen demand (COD) of the paddy water was 342 mg/L in paddy water, but the COD was 8 and 72 mg/L in the reservoir and pond waters, respectively [27]. In the presence of OPC, photolysis of CTL preferred in distilled water and reservoir water. The possible reason was that dissolved organic matter (DOM) in the paddy and pond water, such as humic substances, could react with the excited triplet states of CTL [28]. Some anions, e.g., Cl^-, CO_3^{2-} and HCO_3^-

could also react with the excited triplet states which braked the photolysis rate [29,30].

4. Conclusions

OPC significantly enhanced CTL degradation and avoided the formation of the highly toxic 4-OH-CTL. The 96-h LC_{50} values of the photodegradation product showed 252 and 5.5 fold more than CTL and 4-OH-CTL on zebrafish. Therefore, CTL should be photodegraded faster using OPC as an enhancer to clean up CTL pollution *via* the sunlight photodegradation. The study of the mechanism of phototransformation of CTL revealed that photolysis is favored at both oxygen free condition in the presence and absence of OPC.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apcatb.2017.05.065.

References

[1] S. Wanwimolruk, O. Kanchanamayoon, K. Phopin, V. Prachayasittikul, Sci. Total Environ. 532 (2015) 447–455.
[2] R.A. Putnam, J.O. Nelson, J.M. Clark, J. Agric. Food Chem. 51 (2013) 170–176.
[3] A. Chaves, D. Shea, D. Danehower, Chemosphere 71 (2008) 629–638.
[4] R.M. Sherrard, J.S. Bearr, C.L. Murray-Gulde, J.H. Rodgers, Y.T. Shah, Environ. Pollut. 127 (2004) 385–394.
[5] D.S. Gamble, A.G. Bruccoleri, E. Lindsay, C.H. Langford, G.A. Leyes, Environ. Sci. Technol. 34 (2000) 125–129.
[6] E.A. Kazos, C.G. Nanos, C.D. Stalikas, C.N. Konidari, Chemosphere 72 (2008) 1413–1419.
[7] K.V. Thomas, M. McHugh, M. Hilton, M. Waldock, Environ. Pollut. 123 (2003) 153–161.
[8] P.E. Davies, R.W.G. White, Aquat. Toxicol. 7 (1985) 93–105.
[9] S.Y. Yu, M.R. Wages, G.P. Cobb, J.D. Maul, Environ. Pollut. 181 (2013) 329–334.
[10] N. Voulvoulis, M.D. Scrimshaw, J.N. Lester, Chemosphere 38 (1999) 3503–3516.
[11] S. Kern, K. Fenner, H.P. Singer, R.P. Schwarzenbach, J. Hollender, Environ. Sci. Technol. 43 (2009) 7039–7046.
[12] S. Monadjemi, M.E. Roz, C. Richard, A.T. Halle, Environ. Sci. Technol. 45 (2011) 9582–9589.
[13] J. Porras, J. Fernandez, R. Torres-Palma, C. Richard, Environ. Sci. Technol. 48 (2014) 2218–2225.
[14] P.-Y. Caux, R.A. Kent, G.T. Fan, G.L. Stephenson, Crit. Rev. Environ. Sci. Technol. 26 (1996) 45–93.
[15] J. Park, S. Lee, I. Rhee, J. Kim, J. Agric. Food Chem. 50 (2003) 7570–7575.
[16] A.C. Affam, M. Chaudhuri, J. Environ. Manag. 130 (2013) 160–165.
[17] V.A. Sakkas, A.A. Albanis, Appl. Catal. B Environ. 46 (2003) 175–188.
[18] A.C. Affam, M. Chaudhuri, S.R. Kutty, K. Muda, Int. Biodeterior. Biodegrad. 93 (2014) 195–201.
[19] Y.Q. Tan, H.X. Xiong, T.Z. Shi, R.M. Hua, X.W. Wu, H.Q. Cao, X.D. Li, J. Tang, J. Agric. Food Chem. 61 (2013) 5003–5008.
[20] J.W. Kwon, K.L. Armbrust, J. Agric. Food Chem. 54 (2006) 3651–3657.
[21] World Health Organization, International Programme on Chemical Safety, 1996. Chlorothalonil. Environmental Health Criteria 183. Geneva, Switzerland.
[22] Y. Tan, Q. Huang, T. Shi, L. Jin, R. Hua, X. Wu, X. Li, H. Cao, J. Tang, Q.X. Li, J. Agric. Food Chem. 62 (2014) 12090–12095.
[23] J.F. Hammerstone, S.A. Lazarus, H.H. Schmitz, J. Nutr. 130 (2000) 2086S–2092S.
[24] F.E. Kandil, L. Song, J.M. Pezzuto, K. Marley, D.S. Seigler, M.A.L. Smith, In Vitro Cell. Dev. Biol. Plant 36 (2000) 492–500.
[25] D. Bagchia, M. Bagchia, S.J. Stohsa, D.K. Dasb, S.D. Rayc, C.A. Kuszynskid, S.S. Joshid, H.G. Pruesse, Toxicology 148 (2000) 187–197.
[26] P. Cos, T. De Bruyne, N. Hermans, S. Apers, D. VandenBerghe, A.J. Vlietinck, Curr. Med. Chem. 11 (2004) 1345–1359.
[27] J. Wenk, U. Von Gunten, S. Canonica, Environ. Sci. Technol. 45 (2011) 1334–1340.
[28] S. Zhang, J. Chen, Q. Xie, J. Shao, Environ. Sci. Technol. 45 (2011) 7945–7946.
[29] A. Jammoul, S. Dumas, B. D'Anna, C. George, Atmos. Chem. Phys. 9 (2009) 4229–4237.
[30] S. Canonica, T. Kohn, M. Mac, F.J. Real, J. Wirz, U. Von Gunten, Environ. Sci. Technol. 39 (2005) 9182–9188.